=> d his

(FILE 'HOME' ENTERED AT 08:51:05 ON 01 MAR 2006)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS, CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB, DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 08:51:13 ON 01 MAR 2006

SEA XYLANASE	
1	FILE ADISCTI
1646	FILE AGRICOLA
33	FILE ANABSTR
79	FILE ANTE
9	FILE AQUALINE
75	FILE AQUASCI
1462	FILE BIOENG
4090	FILE BIOSIS
2579	FILE BIOTECHABS
2579	FILE BIOTECHDS
1496	FILE BIOTECHNO
2230	FILE CABA
7016	FILE CAPLUS
902	FILE CEABA-VTB
30	FILE CIN
132	FILE CONFSCI
13	FILE CROPB
24	FILE CROPU
3	FILE DDFB
9	FILE DDFU
3571	FILE DGENE
164	FILE DISSABS
3	FILE DRUGB
9	FILE DRUGU
26	FILE EMBAL
1632	FILE EMBASE
1699	FILE ESBIOBASE
61	FILE FEDRIP
8	FILE FOREGE
524	FILE FROSTI
1332	FILE FSTA
1933	FILE GENBANK
5	FILE HEALSAFE
611	FILE IFIPAT
572	FILE JICST-EPLUS
1849	FILE LIFESCI
1631	FILE MEDLINE
2	FILE NIOSHTIC
51	FILE NTIS
1	FILE NUTRACEUT
19	FILE OCEAN
2341	FILE PASCAL
26	FILE PHIN
137	FILE PROMT
4	FILE RDISCLOSURE
3793	FILE SCISEARCH
774	FILE TOXCENTER
2160	FILE USPATFULL
210	FILE USPAT2
404	FILE VETU
13	FILE WATER
937	FILE WPIDS
9	FILE WPIFV

```
             937   FILE WPINDEX
L1              QUE XYLANASE
                ---------

     FILE 'CAPLUS, BIOSIS, SCISEARCH, BIOTECHDS, PASCAL, CABA, LIFESCI,
     ESBIOBASE, AGRICOLA, EMBASE, MEDLINE, BIOTECHNO, BIOENG, FSTA' ENTERED AT
     08:52:32 ON 01 MAR 2006
L2         12919 S L1 AND (ISOLAT? OR PURI?)
L3           547 S L2 AND SUBTILIS
L4            14 S L3 AND RESISTANT
L5             5 DUP REM L4 (9 DUPLICATES REMOVED)
L6           269 S L3 AND PY<=1998
L7             3 S L6 AND INHIBITOR
L8             0 S L6 AND (XYLANASE INHIBITOR)
```

L7 ANSWER 1 OF 3 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1997:575653 SCISEARCH
THE GENUINE ARTICLE: XN635
TITLE: Production, **purification** and characterization of the hen egg-white lysozyme **inhibitor** from Enterobacter cloacae M-1002
AUTHOR: Wang S L (Reprint); Pai C S; Shieh S T
CORPORATE SOURCE: DA YEH INST TECHNOL, DEPT FOOD ENGN, CHANGHUA, TAIWAN (Reprint)
COUNTRY OF AUTHOR: TAIWAN
SOURCE: JOURNAL OF THE CHINESE CHEMICAL SOCIETY, (**JUN 1997**) Vol. 44, No. 3, pp. 349-355.
ISSN: 0009-4536.
PUBLISHER: CHINESE CHEM SOC, PO BOX 609, TAIPEI 10099, TAIWAN.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: PHYS
LANGUAGE: English
REFERENCE COUNT: 36
ENTRY DATE: Entered STN: 1997
Last Updated on STN: 1997

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Three hen egg-white lysozyme **inhibitor** producing strains, Enterobacter cloacae M-1002, E. sakazakii M-1204, and Erwinia rhapontici H-55, were **isolated** from the soils of Taiwan. E. cloacae M-1002 appeared to be a promising **inhibitor** producing strain. One **inhibitor** was **isolated** from the culture broth of this strain. Maximum lysozyme inhibitory activity was obtained when the bacterium was grown aerobically in a medium consisting of 0.75% glucose, 0.25% beef extract, 1.0% polypeptone, and 0.25% sodium L-glutamate (pH 7.0) at 37 degrees C after 36-48 hrs. A hen egg-white lysozyme **inhibitor** was **isolated** from the culture broth of this strain. The **inhibitor** was **purified** from the culture supernatant of E. cloacae M-1002 by ammonium sulfate fractionation, DEAE-Sepharose CL-6B column chromatography and Fractogel TSK HW-55 (S) gel chromatography. Molecular weight of the **purified** lysozyme **inhibitor** was estimated to be 18,000-20,000 by SDS-PAGE and HPLC, and was composed of 71% amino acid and 23% total sugar. Serine, glycine, and alanine in a 3:2:1 molar ratio were the major amino acids, calculated to be 32.8, 20.3, and 11.4% (mol%), respectively. Glucose and mannose were the major sugar components of the **inhibitor**. The **inhibitor** was stable at pH 5 to 8 and was stable under 50 degrees C. Only hen egg-white lysozyme was inhibited by the **purified inhibitor** but not the other tested enzymes such as lysozyme of celery, turnip; lytic enzyme of Pseudomonas aeruginosa M-1001; chitinase/lysozyme of P. aeruginosa K-187; or cellulase and **xylanase** of Streptomyces actuosus A-151 and Aspergillus sp. G-393. The inhibition of lysozyme to the bacterial cell lyric activity by the **purified inhibitor** was 100%.

L7 ANSWER 2 OF 3 SCISEARCH COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 1996:44422 SCISEARCH
THE GENUINE ARTICLE: TN226
TITLE: **Purification** and characterization of two arabinofuranosidases from solid-state cultures of the fungus Penicillium capsulatum
AUTHOR: Filho E X F (Reprint); Puls J; Coughlan M P
CORPORATE SOURCE: UNIV BRASILIA, DEPT BIOL CELULAR, LAB ENZIMOL, BR-70910900 BRASILIA, DF, BRAZIL (Reprint); INST HOLZCHEM, HAMBURG, GERMANY; NATL UNIV IRELAND UNIV COLL GALWAY, DEPT BIOCHEM, GALWAY, IRELAND

COUNTRY OF AUTHOR: BRAZIL; GERMANY; IRELAND
SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, **(JAN 1996***)**
Vol. 62, No. 1, pp. 168-173.
ISSN: 0099-2240.
PUBLISHER: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 46
ENTRY DATE: Entered STN: 1996
Last Updated on STN: 1996
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB **Two arabinofuranosidases, termed Ara I and Ara II, from solid-state cultures of Penicillium capsulatum were ***purified** to apparent homogeneity as judged by electrophoresis and isoelectric focusing. Each enzyme is a single subunit glycoprotein, and they have M(r)s and pIs of 64,500 and 4.15 (Ara I) and 62,700 and 4.54 (Ara II), respectively. Ara I is most active at pH 4.0 and 60 degrees C, while Ara II exhibits optimal activity at pH 4.0 and 55 degrees C. Ara I is the more thermostable, with its half-life at 70 degrees C and pH 4.0 being 17.5 min. ny contrast. the half-life of Ara II is only 9 min at 60 degrees C and pH 4.0. Ara I has the lower K-m and higher catalytic constant values with p-nitrophenyl-alpha-L-arabinofuranoside being used as the substrate. Arabinose, a competitive **inhibitor** (K-i, 16.4 mM) of Ara II, has no effect on Ara I activity at concentrations of up to 40 mM. Each enzyme catalyzes the release of arabinose from pectin, araban, and certain arabinose-containing xylans. The last activity is enhanced by pretreatment of the relevant substrates with **xylanase**, ferulic acid esterase, or combinations of these enzymes. Thus, arabinoxylooligosaccharides in which arabinose is the sole side chain substituent appear to be the preferred substrates. On the basis of the evidence cited above, each enzyme has been classified as an alpha-L-arabinofuranoside arabinofuranohydrolase (EC 3.2.1.79).

L7 ANSWER 3 OF 3 MEDLINE on STN
ACCESSION NUMBER: 94271752 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7911679
TITLE: Identification of glutamic acid 78 as the active site nucleophile in Bacillus **subtilis xylanase** using electrospray tandem mass spectrometry.
AUTHOR: Miao S; Ziser L; Aebersold R; Withers S G
CORPORATE SOURCE: Department of Chemistry, University of British Columbia, Vancouver, Canada.
SOURCE: Biochemistry, **(1994 Jun 14)** Vol. 33, No. 23, pp. 7027-32.
Journal code: 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199407
ENTRY DATE: Entered STN: 19940729
Last Updated on STN: 19970203
Entered Medline: 19940721

AB A new mechanism-based inactivator of beta-1,4-**xylanases**, 2',4'-dinitrophenyl 2-deoxy-2-fluoro-beta-xylobioside, has been synthesized and used to trap the covalent intermediate formed during catalysis by Bacillus **subtilis xylanase**. Electrospray mass spectrometry confirmed the 1:1 stoichiometry of the incorporation of inactivator into the enzyme. Inactivation of **xylanase** followed the expected pseudo-first-order kinetic behavior, and kinetic parameters were determined. The intermediate trapped was relatively stable toward hydrolytic turnover (t1/2 = 350 min). However, turnover could be

facilitated by transglycosylation following the addition of the acceptor benzyl thio-beta-xylobioside, thus demonstrating the catalytic competence of the trapped intermediate. Reactivation kinetic parameters for this process of kre = 0.03 min-1 and Kre = 46 mM were determined. The nucleophilic amino acid was identified as Glu78 by a tandem mass spectrometric technique which does not require the use of radiolabels. The peptic digest of the labeled enzyme was separated by high-performance liquid chromatography and the eluent fed into a tandem mass spectrometer via an electrospray ionization device. The labeled peptide was identified as one of m/z = 826 (doubly charged) which fragmented in the collision chamber between the mass analyzers with loss of the mass of a 2-fluoroxylobiosyl unit. Confirmation of the peptide identity was obtained both by tandem mass spectrometric sequencing and by Edman degradation of the **purified** peptide. Glu78 is completely conserved in all members of this **xylanase** family and indeed is shown to be located in the active site in the recently determined X-ray crystal structure.

=>

=> d l5 ibib ab 1-5

L5 ANSWER 1 OF 5 MEDLINE on STN
ACCESSION NUMBER: 2005464862 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16131137
TITLE: Enzymic degradability of hull-less barley flour alkali-solubilized arabinoxylan fractions by endoxylanases.
AUTHOR: Trogh Isabel; Croes Evi; Courtin Christophe M; Delcour Jan A
CORPORATE SOURCE: Laboratory of Food Chemistry, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium.. isabel.trogh@biw.kuleuven.be
SOURCE: Journal of agricultural and food chemistry, (2005 Sep 7) Vol. 53, No. 18, pp. 7243-50.
Journal code: 0374755. ISSN: 0021-8561.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200510
ENTRY DATE: Entered STN: 20050901
Last Updated on STN: 20051012
Entered Medline: 20051011

AB The impacts of the arabinose to xylose (A/X) ratio of arabinoxylans (AX) and the endoxylanase substrate specificity on the enzymic degradability of hull-less barley flour AX by endoxylanases were studied by using alkali-solubilized AX (AS-AX) fractions with different A/X ratio, on the one hand, and glycoside hydrolase family 10 and 11 endoxylanases of Aspergillus aculeatus (XAA) and Bacillus **subtilis** (XBS), respectively, on the other hand. AS-AX were obtained by saturated barium hydroxide treatment of hull-less barley flour water-unextractable AX. Fractionation of AS-AX by stepwise ethanol precipitation resulted in structurally different hull-less barley flour AS-AX fractions. Their A/X ratios increased with increasing ethanol concentration, and this increase in A/X ratio was reflected in their xylose substitution levels. For both XAA and XBS, the enzymic degradability of AX and apparent specific endoxylanase activity decreased with increasing A/X ratio of the AS-AX substrates, implying that both endoxylanases were sterically hindered by arabinose substituents. However, for all AS-AX fractions, hydrolysis end products of lower average degree of polymerization were obtained after incubation with XAA than with XBS, indicating that the former enzyme has a lower substrate specificity toward hull-less barley flour AS-AX than the latter. In addition, apparent specific endoxylanase activities indicated that XBS was approximately 2 times more sensitive to variations in the A/X ratio of AS-AX fractions than XAA. Furthermore, AS-AX with higher A/X ratio were relatively **resistant** to degradation by XBS.

L5 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-25655 BIOTECHDS
TITLE: Construction, expression, and characterization of a thermostable **xylanase**;
production and characterization of a thermostable **xylanase** useful as a food-additive
AUTHOR: WENG XY; SUN JY
CORPORATE SOURCE: Zhejiang Univ; Zhejiang Univ
LOCATION: Sun JY, Zhejiang Univ, Coll Anim Sci, Div Microbiol, Feed Sci Inst, Hangzhou 310029, Peoples R China
SOURCE: CURRENT MICROBIOLOGY; (2005) 51, 3, 188-192
ISSN: 0343-8651
DOCUMENT TYPE: Journal
LANGUAGE: English

AB AUTHOR ABSTRACT - A hybrid gene, btx, encoding a thermostable

xylanase, Btx, was constructed by substituting the 31 N-terminal amino acid residues of the Thermomonospora fusca **xylanase** A (TfxA) for the corresponding region of 22 amino acid residues of the Bacillus **subtilis xylanase** A (BsxA). The btx gene was expressed in Escherichia coli BL21. The halo size produced by **xylanase** Btx on a Remanzol brilliant blue R (RBB) xylan plate at 60 degrees C and pH 6.0 was larger than those of BsxA and TfxA. The molecular weight of Btx was 22 kDa. Temperature and pH optima for Btx were at 50-60 degrees C and 6.0, respectively. Btx showed activity over 80% over a pH range of 5.0-9.0, which was wider than that of BsxA, and was also more acid-**resistant** than TfxA. Btx exhibited significant thermostability compared with BsxA. The results show the importance of the N-terminal sequence of TfxA in thermostability. (5 pages)

L5 ANSWER 3 OF 5 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-25262 BIOTECHDS
TITLE: High-level expression, **purification**, and characterization of recombinant wheat **xylanase** inhibitor TAXI-I secreted by the yeast Pichia pastoris; recombinant enzyme-inhibitor protein production via plasmid expression in host cell
AUTHOR: FIERENS K; GEUDENS N; BRIJS K; COURTIN CM; GEBRUERS K; ROBBEN J; VAN CAMPENHOUT S; VOLCKAERT G; DELCOUR JA
CORPORATE SOURCE: Katholieke Univ Leuven; Katholieke Univ Leuven
LOCATION: Fierens K, Katholieke Univ Leuven, Food Chem Lab, Kasteelpk Arenberg 20, B-3001 Heverlee, Belgium
SOURCE: PROTEIN EXPRESSION AND PURIFICATION; (2004) 37, 1, 39-46
ISSN: 1046-5928
DOCUMENT TYPE: Journal
LANGUAGE: English
AB AUTHOR ABSTRACT - Triticum aestivum **xylanase** inhibitor I (TAXI-I) is a wheat protein that inhibits microbial **xylanases** belonging to glycoside hydrolase family 11. In the present study, recombinant TAXI-I (rTAXI-I) was successfully produced by the methylotrophic yeast Pichia pastoris at high expression levels (similar to75 mg/L). The rTAXI-I protein was **purified** from the P. pastoris culture medium using cation exchange and gel filtration chromatographic steps. rTAXI-I has an iso-electric point of at least 9.3 and a mass spectrometry molecular mass of 42,013 Da indicative of one N-linked glycosylation. The recombinant protein fold was confirmed by circular dichroism spectroscopy. **Xylanase** inhibition by rTAXI-I was optimal at 20-30degreesC and at pH 5.0. rTAXI-I still showed **xylanase** inhibition activity at 30degreesC after a 40 min pre-incubation step at temperatures between 4 and 70degreesC and after 2 h pre-incubation at room temperature at a pH ranging from 3.0 to 12.0, respectively. All tested glycoside hydrolase family 11 **xylanases** were inhibited by rTAXI-I whereas those belonging to family 10 were not. Specific inhibition activities against family 11 Aspergillus niger and Bacillus **subtilis xylanases** were 3570 and 2940 IU/mg protein, respectively. The obtained biochemical characteristics of rTAXI-I produced by P. pastoris (no proteolytical cleft) were similar to those of natural TAXI-I (mixture of proteolytically processed and non-processed forms) and non-glycosylated rTAXI-I expressed in Escherichia coli. The present results show that **xylanase** inhibition activity of TAXI-I is only affected to a limited degree by its glycosylation. or proteolytic, processing. (C) 2004 Elsevier Inc. All rights reserved.
DERWENT ABSTRACT: The TAXI-I **xylanase** inhibitor gene was **isolated** from the E. coli pBAD/Thio TAXI-I expression vector using restriction digestion with Bg/II. The TAXI-I coding sequence was cloned into the BsmBI site of the Pichia pastoris pPICZotC secretion vector. Proper insert orientation was tested by restriction digestion and

sequencing. Sequencing reactions were performed with 5'AOX and 3'AOX vector-specific primers, and reaction products were analyzed on a 377 DNA Sequencer using ABI PRISM Big Dye Terminator chemistry. The recombinant (pPICZotC-TAXI-1) expression vector was propagated in the Escherichia coli TOP10 strain. Plasmid DNA was **isolated** and linearized with PmeI. The digested DNA was used for transformation of the P. pastoris X33 strain according to the EasyComp Transformation protocol. Genomic DNA of Zeocin-**resistant** P. pastoris transformants was **isolated** and the presence of the TAXI-I gene was determined by PCR using vector-specific primers and HotStartaq DNA polymeras(8 pages)

L5 ANSWER 4 OF 5 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2002-12004 BIOTECHDS
TITLE: Novel variant of cell-wall degrading enzyme having beta-helix structure, specifically variant of wild-type pectate lyase useful in textile, detergent and cellulose fiber processing and in wine and juice processing;
plasmid-pMB54-mediated recombinant pectate-lyase, alpha-amylase, chloramphenicol-acetyltransferase fusion protein gene transfer and expression in Bacillus **subtilis** and transgenic plant for use as a feed-addictive and in thepaper industry
AUTHOR: SCHUELEIN M; GLAD S O S; ANDERSEN C; FRANDSEN T P
PATENT ASSIGNEE: NOVOZYMES AS
PATENT INFO: WO 2002006442 24 Jan 2002
APPLICATION INFO: WO 2000-DK505 19 Jul 2000
PRIORITY INFO: DK 2001-734 10 May 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-241511 [29]
AB DERWENT ABSTRACT:
NOVELTY - An improved variant of a cell-wall degrading enzyme having a beta-helix structure, in particular a variant (I) of a wild-type parent pectate lyase (EC 4.2.2.2) having the conserved amino acid residues D111, D141 or E141, D145, K165, R194 or R199 when aligned with the pectate lyase, comprising a sequence of 302 amino acids defined in the specification, is new.

DETAILED DESCRIPTION - The improved variant of a cell-wall degrading enzyme, which holds a substituent in a position determined, by: (a) identifying all residues potentially belonging to a stack, (b) characterizing the stack as interior or exterior, (c) characterizing the stack as polar (typically R, S, or T) or hydrophobic (either aliphatic L, I or V; or aromatic/heteroaromatic F, Y, H, W) based on the dominating characteristics of the parent or wild-type enzyme stack residues and/or its orientation relative to the beta-helix (interior or exterior), (d) optimizing all stack positions of a stack either to hydrophobic aliphatic amino acids, hydrophobic aromatic amino acids (preferably H alone, Y and F alone or in combination) or polar amino acids (preferably R) by allowing mutations within one or all positions to amino acids belonging to one of these groups, (e) measuring thermostability of the variants by disc scanning calorimetry (DSC) or an application-related assay such as a Pad-Steam application test, and (f) selecting the stabilized variants. (I) is substituted in at least one position chosen from the positions 5, 8, 9, 10, 19, 38, 39, 40, 41, 55, 56, 59, 61, 64, 71, 72, 82, 83, 90, 100, 102, 109, 112, 114, 117, 129, 133, 136, 137, 139, 142, 144, 160, 163, 164, 166, 167, 168, 169, 171, 173, 179, 189, 192, 197, 198, 200, 203, 207, 214, 220, 222, 224, 230, 232, 236, 237, 238, 244, 246, 261, 262, 264, 265, 266, 269, 278, 282, 283, 284, 285, 288, 289 and 297. INDEPENDENT CLAIMS are also included for the following: (1) providing an improved variant of a cell-wall degrading enzyme having a beta-helix structure, by performing (a)-(f) as stated above; (2) an **isolated** polynucleotide molecule (II) encoding (I), prepared from the molecule comprising the DNA sequence of 909 bp defined in the specification by

conventional methods such as site-directed mutagenesis; (3) an expression vector (III) comprising operably linked (II), a transcription promoter, degenerate sequence of (II) or promoter, and a transcription terminator; (4) a culture cell (IV) containing (III) and expressing the polypeptide encoded by the DNA segment; (5) producing a polypeptide having pectate lyase activity; (6) an **isolated** enzyme having pectate lyase activity, in which the enzyme is free from homologous impurities and is produced by the above method; (7) an enzyme preparation (V) comprising (I); and (8) a detergent composition comprising (I) or (V).

WIDER DISCLOSURE - Transgenic plant part or plant cell (or its progeny) which has been transformed with (II) is also disclosed.

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (IV) and recovering the polypeptide. Preferred Polypeptide: (I) is derived from a wild-type variant holding the conserved amino acid residues W123, D125 and H126. Preferred Composition: (V) further comprises one or more enzymes such as proteases, cellulases (endoglucanases), beta-glucanases, hemicellulases, lipases, peroxidases, laccases, cutinases, pectinases, reductases, oxidases, phenoloxidases, ligninases, **xylanases**, pectin acetyl esterases, or their mixtures.

USE - (I) or enzyme preparation containing (I) is useful for improving the properties of cellulosic fibers, yarn, woven or non-woven fabric, in which the enzyme preparation or the enzyme is used in a scouring process, and also for degradation or modification of plant material including recycled waste paper, mechanical paper-making pulps or fibers subjected to a retting process (claimed). (I) or enzyme preparation containing (I) is useful for degradation or modification of plant cell walls or any pectin-containing material originating from plant cell wall and for separation of components of plant cell materials, e.g sugar or starch rich plant material into components of commercial interest like sucrose from sugar beet or starch from potato. Also facilitates separation of protein-rich or oil-rich crops into valuable protein and oil and invaluable hull fractions. (I) can be used alone or together with glucanases, pectinases and/or hemicellulases to improve the extraction of oil from oil-rich plant material, like soy-bean, olives, rape-seed or sunflower. (I) is also useful in the preparation of fruit or vegetable juice to increase yield, and in the enzymatic hydrolysis of various plant cell wall-derived materials or waste materials, e.g. from wine or juice production, or agricultural residues such as vegetable bean hulls, sugar beet pulp, olive pulp and potato pulp. (I) is further useful in modifying the viscosity of plant cell wall derived material, in vegetable or fruit juice, especially in apple or pear juice, and is useful in animal feed additive to improve the in vivo breakdown of plant cell wall material. (I) or enzyme preparation containing (I) is useful for treatment of mash from fruits and vegetables to improve the extractability or degradability of the mash. For e.g. in the treatment of mash from apples and pears for juice production, and in mash treatment of grapes for wine production.

ADVANTAGE - (I) exhibits increased thermostability compared to parent enzyme.

EXAMPLE - The wild-type Bacillus licheniformis pectate lyase encoded by a sequence of 909 bp defined in the specification, was expressed in B. **subtilis** from a plasmid denoted pMB541. This plasmid contained a fusion of the signal sequence from B.licheniformis alpha-amylase and the gene encoding the mature protein of B.licheniformis pectate lyase, the expression of which was directed by the B.licheniformis alpha-amylase promoter. Further, the plasmid contained the origin of replication, ori, from plasmid pUB110 and the cat (chloramphenicol acetyl transferase) gene from plasmid pC194 conferring resistance towards chloramphenicol. A specific mutagenesis vector with a 1.2 kb pUC fragment inserted in the unique PstI restriction site located between the nucleotide sequence coding for the signal sequence and the mature, was prepared. This vector, denoted pCA134 included an origin of replication derived from the pUC plasmids, the cat gene conferring resistance towards chloramphenicol and

gene coding the mature part of the wild-type B.licheniformis pectate lyase. After verification of the DNA sequence in variant plasmids, the PstI-PstI fragment from pUC was removed and the remaining part of the vector was ligated and transformed into the protease- and amylase-depleted B.**subtilis** strain SHA273, in order to express the variant enzyme. To improve the stability of the pectate lyase site-directed mutagenesis was carried out using the mega-primer method as described by Sarkar and Sommer, 1990, BioTechniques 8:404-407. The B.licheniformis pectate lyase variant M169I+F198V was constructed by the use of the gene specific primer Pely01 (5'-CGACTGGCAATGCCGGGGCGG-3') and mutagenic primers Pely22 (GGAAATCAATGCTGATCGGTTCATCGGACAGC) and Pely23 (CGTGTGCCGTCAGTACGTTTCGGAGGAGGC) to amplify by PCR a 470 bp DNA fragment from the pCA134 plasmid. The 470 bp fragment was **purified** and used as a mega-primer together with primer 113711 (GAAACAGCTATGACCATGATTACGCC) in a second PCR carried out on the same template. The resulting 1050 bp fragment was digested with restriction enzymes BclI and NotI and the resulting 570 bp DNA fragment was **purified** and ligated with the pCA134 plasmid digested with the same enzymes. Competent B.**subtilis** SHA273 cells were transformed with the ligation, and chloroamphenicol **resistant** transformants were checked by DNA sequencing to verify the presence of the correct mutations on the plasmid. The activity of the pectate lyase variant M169I+F198V, in textile preparation was determined by measuring the amount of pectin removed from the fabric after treating with the enzyme in a surfactant-buffer solution. The results clearly indicated that the pectate lyase variant performed better at lower dosages than the wild-type pectate lyase. The maximum average pectin removal observed for pectate lyase in general was 30%, an excellent scouring effect on cotton. (158 pages)

L5 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2006 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 1993:226821 CAPLUS
DOCUMENT NUMBER: 118:226821
TITLE: Molecular cloning and expression of **xylanases** from an alkalophilic thermophilic Bacillus (NCIM 59) in Bacillus **subtilis** A8
AUTHOR(S): Shendye, Abhay; Rao, Mala
CORPORATE SOURCE: Div. Biochem. Sci., Natl. Chem. Lab., Pune, 411 008, India
SOURCE: Enzyme and Microbial Technology (1993), 15(4), 343-7
CODEN: EMTED2; ISSN: 0141-0229
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The 6.5-kb Hind III fragment of alkalophilic-thermophilic Bacillus genomic DNA, coding for two **xylanases**, was cloned from Escherichia coli recombinant plasmid pATB X235 in a Bacillus plasmid pLP1202 at the Hind III site, inactivating the tetracycline resistance gene. Bacillus **subtilis** A8 was transformed with the ligation mixture using electroporation. The recombinants were chloroamphenicol-**resistant**, tetracycline-sensitive, and showed clearance on LB plates having xylan and immunol. cross-reactivity with the antibodies raised against the **purified xylanase** (Mr 15,800) from alkalophilic-thermophilic Bacillus. The **xylanase** activity obtained in B. **subtilis** A8 was fivefold higher than in E. coli harboring pATB X235. Ninety-five percent of the enzyme activity was extracellular. The **xylanases** produced by the recombinant showed mol. wts. of 35 and 14.5 kDa. The hydrolysis of xylan by the recombinant **xylanases** yielded mainly xylobiose. Xylose was also detected, along with traces of xylotriose and xylotetrose.

=> d his